Enhancing Immunity Against Melanoma using Type I Interferon

by

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Declaration

I declare this thesis is my own account of my research and contains as its main content, work which has not been previously submitted for a degree at any tertiary educational institution.

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Word Count

Abstract:	292
1. Introduction:	6,366
2. Materials and methods:	3,674
3. Results:	6,460
4. Discussion:	4,870
5. Conclusion:	580
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Abstract

Melanoma is an aggressive cancer, which when diagnosed in the late stages has a median survival rate of 6 months. The current gold-standard for adjuvant therapy is high doses of the type I interferon (IFN)-α2, which prolongs disease free survival, but not overall survival. This high-dose therapy has many serious side-effects, resulting in doses to be lowered to sub-therapeutic levels. It is not clear how IFN-α2 acts to treat melanoma, however it is accepted that immune enhancement is involved. The two main type I IFN families, IFN-α and IFN-β, signal via the same receptor, however they have been found to be functionally different. Of the IFN-α family, 13 subtypes have been discovered and there is evidence that each is functionally unique. This project explores the therapeutic efficacy of seven type I IFN subtypes, including IFN-α2 and IFN-β, in treating melanoma. To test efficacy, a model of DNA therapy to administer plasmids encoding the IFN subtypes was combined with a melanoma model utilizing B16 F1 cells expressing the immunogenic peptide glycoprotein B. We have found that the IFN-α subtypes tested have greater efficacy than IFN-α2 in treating melanoma. The IFN subtypes delayed tumour onset, and one subtype demonstrated a significant increase in survival time. In addition, therapeutic effects could be seen when sera levels were lower than that of IFN-α2. The *in vitro* effects of the IFN subtypes on melanoma cells was examined, uncovering differences in potency. Furthermore, we have found evidence of anti-tumour immune enhancement is associated with IFN-α therapy but not IFN-β. This research shows that the IFN-α subtypes differ in their anti-tumour efficacy, and potency of actions. Therefore the IFN-α subtypes show potential as a superior therapy in treating melanoma, which may be administered at lower, less toxic doses.

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Abbreviations

ACT	Adoptive cell therapy
APC	Antigen presenting cell
B6	C57Bl/6J
Bcl-2	B-cell lymphoma 2 (gene)
BP	Bupivacaine
BSA	Bovine serum albumin
CFSE	Carboxyfluorescein succinimidyl ester
CpG	Cytosine—phosphate—Guanine (linear sequence)
CTL	Cytotoxic T-lymphocyte
CTLA-4	Cytotoxic T-lymphocyte antigen 4
DC	Dendritic cell
DNA	Deoxyribose nucleic acid
dsRNA	Double stranded ribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
FCS	Foetal calf serum
FSC	Forward scatter
gB	Glycoprotein B
GFP	Green fluorescent protein
HEPES	hydroxyethyl piperazineethanesulfonic acid
HLA	Human leukocyte antigen (human MHC)
HSV	Herpes simplex virus
IFN	Interferon
IFN-I	Type I interferon
IFNAR	Type I IFN receptor
IgG	Immunoglobulin G
IL	Interleukin
IRES	Internal ribosome entry site
ISGs	Interferon stimulated genes

IU	International units
i.v.	intravenous
JAK	Janus Kinase
LB	Lubia Broth
LN	Lymph node
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinases
MHC	Major Histocompatibility Complex
MS	Multiple Sclerosis
MU	Million units
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer
PAMP	Pathogen associated molecular pattern
PBS	Phosphate buffered saline
PI	Propidium Iodide
PRR	Pattern recognition receptors
RAG	Recombination activating gene
RGP	Radial growth phase
RPMI	Roswell Park Memorial Institute medium
SAV	Streptavidin
SN	Supernatant
SSC	Side scatter
ssRNA	Single stranded ribonucleic acid
STAT	Signal transducers and activators of transcription
TA	Tibialis anterior
TAE	Tris-acetate-EDTA
TLR	Toll like receptor
TRAIL	TNF-related apoptosis-inducing ligand
Tyk2	Tyrosine Kinase 2
VGP	Vertical growth phase
UV	Ultraviolet (light)

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